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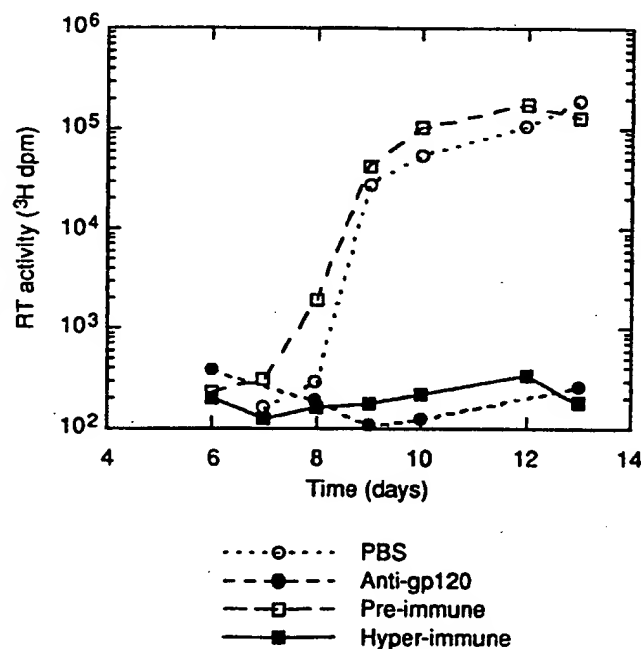
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<p>(21) International Application Number: PCT/GB93/01503 (22) International Filing Date: 16 July 1993 (16.07.93) (30) Priority data: - 9215129.9 16 July 1992 (16.07.92) GB (71) Applicant (for all designated States except US): MEDICAL RESEARCH COUNCIL [GB/GB]; 20 Park Crescent, London W1N 4AL (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : BUTLER, Peter, Jonathan, Gasking [GB/GB]; 2 Heron's Close, Cambridge CB2 4NS (GB). HACKING, Graeme, Norman, Varey [GB/GB]; Selwyn College, Cambridge CB3 9DQ (GB). (74) Agent: KEITH W. NASH & CO.; Pearl Assurance House, 90-92 Regent Street, Cambridge CB2 1DP (GB).</p>		<p>(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. With amended claims.</p>

(54) Title: PEPTIDES THAT MIMIC gp120 HIV EPI TOPE

(57) Abstract

The invention provides a molecule comprising a peptide having the amino acid sequence lysine-proline-cysteine-valine-lysine-leucine-threonine-proline-leucine-cysteine-valine (Seq.Id.No.1), wherein each cysteine residue is disulphide bridged to a further cysteine residue or is derivatised to simulate part of a disulphide bond, or functionally equivalent variants of such a peptide which mimic the immunogenic behaviour of an epitope of gp120 of HIV env. The sequence is highly conserved, being totally conserved in all listed isolates of HIV-1 and HIV-2, and undergoes only minor changes in isolates of SIV. With each cysteine residue contributing to a respective disulphide bridge (actual or simulated by suitable derivatisation), the molecule accurately mimics behaviour of the corresponding sequence of gp120 and elicits an immune response. The molecule can thus be used as the basis of a potential vaccine against AIDS and AIDS related conditions, and may find use in the treatment of AIDS and related conditions.



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Title PEPTIDES THAT MIMIC GP120 HIV EPITOPE.

Field of the Invention

This invention concerns developments relating to human immunodeficiency viruses (HIV).

Background to the Invention

The envelope glycoprotein (env) of human immunodeficiency virus (HIV) is highly variable between independent isolates (reference 1) and this variation is even more marked between HIV-1 and HIV-2(2). This variation is seen not only between independent isolates, but also for sequential isolates of virus from an individual patient (3, 4). Not surprisingly, similar sequence variation has been observed between the human and simian immunodeficiency viruses (SIV) and also within SIV even when isolated from the same species of simian (5-7).

Not only is the variation greater in the envelope glycoprotein than in other gene products of HIV, but even within this one protein the variation is concentrated into specific variable regions (mostly in the surface portion (gp120) generated by the proteolytic maturation of the initial gene product), with other regions being less variable (8, 9). Unfortunately the most variable regions are often also the most immunogenic (10), so that the virus partially evades the host's immune response and

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establishes a persistent infection.

This variability presents problems for diagnostic techniques based upon specific interactions, with separate or mixed reagents usually being employed to test samples for both HIV-1 and HIV-2. This variability also presents problems for any possible vaccine or immune therapy, since any suitable agent will have to give a response towards the many variant virus strains as well as towards HIV-1 and HIV-2.

The present invention is based on the discovery by the inventors of a hitherto unrecognised highly conserved undecapeptide in gp 120 of env, starting from position 122 (on our alignment of HIV-1 env sequences), which is present in HIV-1, HIV-2 and also SIV. This sequence is totally conserved as Lys-Pro-Cys-Val-Lys-Leu-Thr-Pro-Leu-Cys-Val (Seq. Id. No. 1, also referred to as sequence A) in all listed isolates of both types of HIV, including the "highly divergent" HIV-2 isolates D194 and D205 (11), and only undergoes conservative changes at the threonine and second valine in all isolates of SIV except in the highly divergent African mandrill isolate (12) wherein threonine and both leucines are mutated.

In this specification amino acids are either identified by their full names, by conventional 3 letter abbreviations or by conventional 1 letter symbols, as listed in Table 1.

Table 2 gives amino acid sequence data for a number of different isolates of HIV-1, HIV-2 and SIV compared with the highly conserved consensus sequence. The consensus sequence is based upon an absolute majority; for

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individual isolates a dash indicates agreement with the consensus, otherwise the amino acid is shown.

Comparison of the nucleotide sequences in the gene coding for this peptide also shows a high degree of conservation, although the redundancy of the genetic code allows slightly wider variation.

Sequence information was taken from the Los Alamos Data Base.

Earlier reports have shown that the immunodominant regions of gp120 are largely in the variable loops, particularly V3 (e.g. 10). Limited studies with peptides have included the conserved region we describe (e.g. 13). These peptides showed very little immunogenicity. This is now thought to be because no precautions were taken to protect the amino acid side chains from modification during conjugation to the carrier protein.

The conserved sequence identified above is referred to in EP 0298633 of Proteus Biotechnology Limited as the possible basis of a vaccine to promote immunity against at least one strain of HIV. The sequence is stated to have been chosen on the basis of its topographical similarity to at least one other antigenic determinant of the HIV envelope proteins. However, there is no indication in EP 0298633 of an appreciation of the high degree of conservation of the sequence between different strains of HIV-1, HIV-2 and SIV.

EP 0298633 also discloses linking the C terminal of the conserved sequence identified above to a further sequence chosen in similar manner, and it is stated in this

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document that the inessential C terminal dipeptide Cys-Val may possibly be omitted, and that the first Cys residue of the sequence may be cross linked to another Cys residue in the molecule via an intramolecular disulphide bridge.

The present invention is further based on the appreciation that correct presentation of the conserved sequence is very important for eliciting an immune response. It is now known that in its native form the conserved sequence identified above is cross-linked via intramolecular disulphide bridges to a further sequence of gp120 different from that proposed in EP 0298633. This further sequence (sequence B) is also highly conserved within strains of both HIV-1 and HIV-2, with only limited mutations between the two types. SIV isolates show strong similarities to either HIV-1 (SIV_{CPZ}) or HIV-2 (all other strains of SIV) (Table 3). It is further now appreciated that both cysteine residues of the conserved sequence identified above are involved in respective disulphide bridges in the native confirmation (14), and it is believed that both cysteine residues are required to elicit a proper immune response, contrary to the statement in EP 0298633 that one of the cysteine residues is inessential. The strong conservation of this second peptide is fully compatible with it forming an essential structural conformation together with the conserved sequence.

In order to demonstrate this, the present inventors have carried out work using the conserved sequence identified above with both cysteine residues derivatised to approximate to one half of a disulphide bond, thus mimicking native presentation. Initial immunogenic studies on this peptide show that the peptide is

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immunogenic when presented coupled to an appropriate carrier protein and that the resulting antisera react with native gp 120 in permeabilised cells. Work has been carried out using the conserved sequence identified above linked to a further sequence via disulphide bridges from both cysteine residues of each sequence in appropriate orientation.

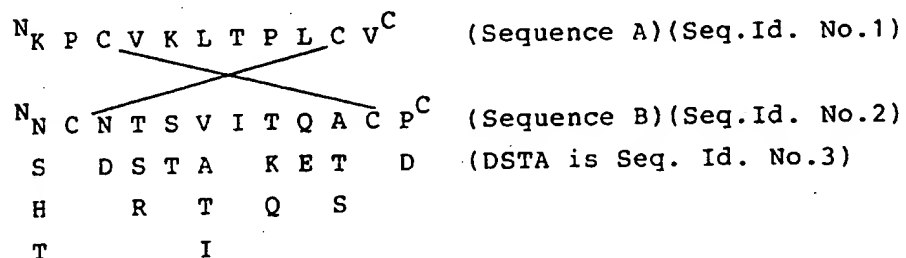
Summary of the Invention

In one aspect the present invention provides a molecule comprising a peptide having the amino acid sequence lysine-proline-cysteine-valine-lysine-leucine-threonine-proline-leucine-cysteine-valine (Seq. Id. No. 1), wherein each cysteine residue is disulphide bridged to a further cysteine residue or is derivatised to simulate part of a disulphide bond, or functionally equivalent variants of such a peptide which mimic the immunogenic behaviour of an epitope of gp120 of HIV env.

The strict conservation of a peptide sequence within the external envelope protein of HIV, coupled with the near conservation in the related SIV, suggests that the sequence that is the basis of the invention is essential for the function of the env-protein and hence unlikely to change further in other, as yet uncharacterised, isolates. Searches have shown that the sequence is not present in other proteins in the EMBL Data Base and so it appears to be specific to immunodeficiency viruses. The invention therefore offers a possible fixed sequence that has many possible implications for the design of diagnostic, immunogenic agents, and therapeutic agents, as will be explained below.

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As explained above, it is important that each cysteine residue contributes to a respective disulphide bridge (actual or simulated by suitable derivatisation) for the molecule to mimic accurately behaviour of the corresponding sequence of gp120. This can be achieved by suitable derivatisation of the cysteine residues, e.g. as the S-acetamidomethyl derivative. This can alternatively be achieved by cross-linking the cysteine residues to respective cysteine residues in a further peptide sequence, which can either be a continuation of the peptide of the invention, spaced with a suitable linker of peptide or other nature, or a separate peptide. One preferred cross-linking arrangement, which is based on the actual cross-linking now known to occur in gp120 is as follows:



Sequence A is the uniquely conserved sequence on which the present invention is based, and Sequence B is based on the sequence of amino acids 219 to 230 of HIV-1 and 210 to 221 of HIV-2, with mutations indicated below in order of frequency. Sequence B is thus fairly conservative although different sequences may be optimal for HIV-1 and HIV-2 (Table 3).

Desired peptide sequences can be readily synthesised in conventional manner, e.g. using Fmoc techniques. Alternatively, peptide sequences can be produced by

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recombinant DNA techniques in known manner.

A further aspect of the invention thus provides a DNA molecule coding for a peptide molecule in accordance with the invention, preferably incorporated into a suitable expression vector.

It will be apparent that the molecule of the invention, based on the highly conserved sequence, can be modified in a variety of different ways without significantly affecting the functionally important immunogenic behaviour of the molecule. Possible modifications to the or each peptide sequence include the following:

1) One or more individual amino acids can be substituted by amino acids having comparable properties e.g. as follows:

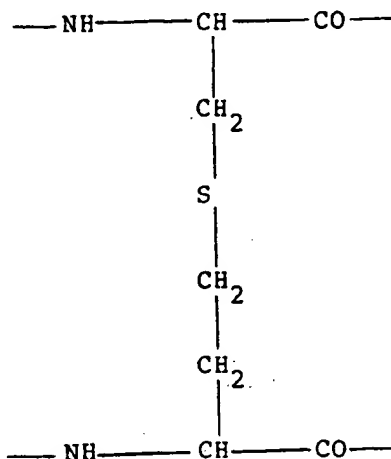
V substituted by I
T substituted by S
K substituted by R
L substituted by I, V or M

2) One or more of the normal peptide linkages can be substituted by isosteric replacements. Possible replacement linkages are well known to those skilled in the art of peptide synthesis and are reviewed, for example, in reference 15. Examples would include: the use of the azapeptide linkage, where the alpha-carbon of the amino acid is replaced by a nitrogen atom (i.e. -NH-NR-CO-); a reduced amide bond, giving a methyl amine (i.e. -NH-CHR-CH₂-); thiomethyl groups, with the CO replaced by CH₂ and the NH by S (i.e. -S-CHR-CH₂-); replacement of both the NH and CO groups with unsaturated carbon atoms (i.e.

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=CH-CHR-CH=); or replacement of the NH group with a methylene (i.e. -CH₂-CHR-CO-).

3) One, or both, pairs of disulphide-bridged cysteine residues (i.e. cystine residues) can be substituted by a cystine analogue such as:



4) One or more of the amino acids can be replaced by a "retro-inverso" amino acid, i.e. a bifunctional amine having a functional group corresponding to an amino acid, as discussed in WO91/13909.

5) One or more non-essential amino acids can be deleted.

6) One or more additional amino acids not significantly affecting function can be included.

7) Structural analogs mimicking the 3-dimensional structure of the peptide can be used in place of the peptide.

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It has been shown that a molecule in accordance with the invention can elicit an immune response. One possible use of the molecule is therefore as the basis of a potential vaccine against AIDS and AIDS related conditions.

In a further aspect the invention thus provides a vaccine against AIDS and AIDS related conditions, comprising a molecule in accordance with the invention.

For this purpose, the molecule of the invention may optionally be linked to a carrier molecule, possibly via chemical groups of amino acids of the conserved sequence or via additional amino acids added at the C- or N-terminus. Many suitable linkages are known, e.g. using the side chains of Tyr residues. Suitable carriers include, e.g., keyhole limpet hemocyanin (KLH), serum albumin, purified protein derivative of tuberculin (PPD), ovalbumin, non-protein carriers and many others. It is thought the lysine residues in the conserved sequence may be of importance in the molecule of the invention, and for this reason the epsilon-amino groups of lysine residues are preferably protected in known manner during a conjugation reaction to link a carrier, to prevent unwanted reaction, with the groups being subsequently regenerated before use of the conjugate.

The molecule of the invention may alternatively be presented as a possible live vaccine, e.g. as part of the coat of a genetically modified virus such as polio or vaccinia.

The vaccine of the invention may be administered in conventional manner, e.g. by injection, orally etc., with or without use of conventional adjuvants such as Freund's

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complete or incomplete adjuvant or aluminium hydroxide, and with or without other immunopotentiating agents. Diluents suitable for use in formulating the vaccine for administration are also well known, including distilled water, phosphate-buffered saline, and buffer solutions such as citrate buffer.

Molecules in accordance with the invention may further find use in the treatment (prophylactic or curative) of AIDS and related conditions, by acting either to displace the binding of the HIV virus to human or animal cells or by disturbing the 3-dimensional organisation of the virus.

A further aspect of the invention thus provides a method for the prophylaxis or treatment of AIDS or related conditions, comprising administering an effective amount of a molecule in accordance with the invention.

In a further aspect, the invention provides a pharmaceutical composition containing, as an active ingredient, a molecule in accordance with the invention, possibly in association with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.

The invention also provides use of a molecule in accordance with the invention for the preparation of a medicament for the therapy or prophylactic treatment of AIDS or related conditions.

Molecules which bind to the conserved sequence on which the invention is based, particularly antibodies, antibody-related molecules and structural analogs thereof, are also of possible use as agents in the treatment and diagnosis

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of AIDS and related conditions.

In a further aspect the invention thus provides a molecule that binds to the conserved sequence that is the basis of the invention, particularly an antibody, an antigen binding site of an antibody or a structural analog thereof.

Techniques for making antibodies (monoclonal and polyclonal) are well known to those skilled in the art.

Variants of antibodies (including an antigen binding site), such as chimeric antibodies, humanised antibodies, veneered antibodies, and engineered antibodies generally are included within the scope of the invention. Techniques for the production of such variants are also well known to those skilled in the art.

With a knowledge of the 3-dimensional structure of the conserved sequence in gp 120, it is possible to design and construct synthetic molecules that will bind to the sequence. Suitable techniques for this purpose are disclosed e.g. in references 16 and 17, which involve the use of computer-modelling to design potential inhibitors to renin.

Antibodies and other molecules which bind to the conserved sequence on which the invention is based can be used for therapeutic (prophylactic and curative) and diagnostic purposes in a number of different ways, including the following:-

- 1) For passive immunisation by suitable administration of antibodies, preferably humanised antibodies to patients.

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- 2) To activate complement or mediate antibody dependent cellular cytotoxicity (ADCC) by use of antibodies of suitable subclass or isotype (possibly obtained by appropriate antibody engineering) to be capable of performing the desired function.
- 3) For targetted delivery of toxins or other agents, e.g. by use of immunotoxins comprising conjugates of antibody and a cytotoxic moiety, for binding directly or indirectly to the target conserved sequence of gp 120.
- 4) For targetted delivery of highly immunogenic materials to the surface of HIV-infected cells, leading to possible ablation of such cells by either the humoral or cellular immune system of the host.
- 5) For detection of HIV, e.g. using a variety of immunoassay techniques.

Techniques for performing all of the above are well known to those skilled in the art.

In another aspect the invention thus covers use of a molecule which binds to the conserved sequence of the invention for therapeutic or diagnostic purposes.

The invention also includes within its scope methods and kits for detecting HIV, antibodies against HIV or infection with HIV using molecules in accordance with the invention.

The invention will be further described, by way of illustration, in the following examples. The first

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example concerns immunological studies on a peptide incorporating the highly conserved sequence, which show that the peptide is immunogenic, as presented, and that the antisera react with native gp 120 in infected cells. The example refers to Figures 1 to 4 of the accompanying drawings, in which:

Figure 1 is a series of photographs showing immunofluorescence of cells, some expressing gp 120 from HIV-1, treated with sera obtained from a rabbit before and after treatment with the peptide, with the serum treated with peptide before application to the cells in some cases;

Figure 2 is a series of photographs showing immunofluorescence of cells, either uninfected or infected with different isolates of HIV-1, treated with sera obtained from a rabbit before and after treatment with the peptide;

Figure 3 is a series of photographs showing immunofluorescence of cells, uninfected or infected, treated with sera obtained from two different rabbits before and after treatment with the peptide; and

Figure 4 is a series of photographs showing immunofluorescence of cells, either uninfected or infected with HIV-2, treated with sera obtained from a rabbit before and after treatment with the peptide.

The second example concerns immunological studies on a peptide incorporating the highly conserved sequence A disulphide-bridged in an antiparallel fashion to the moderately conserved sequence B, which show that the

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peptide is immunogenic, as presented, and that the antiserum reacts with HIV-1 to neutralise its infectivity. The example refers to Figure 5 of the accompanying drawings in which:

Figure 5 is a graph of virus present in the supernatant fluid above tissue culture cells, measured by the activity of viral reverse transcriptase in incorporating ^3H -dTTP, at various times after attempted infection with virus (50 TC1D₅₀) treated for 60 minutes, at 37°C, with the indicated serum (or phosphate buffered saline, PBS) diluted 1:20 with RPMI/10%FCS.

Examples

Analysis of HIV envelope glycoprotein sequences for conservation

HIV and SIV sequences used for the analysis were from the Los Alamos Data Base. Sequence comparisons were performed on a VAX 8600 computer, using HOMED, Version 3.30, from P A Stockwell, Otago University, Dunedin, New Zealand.

Alignment of the sequences of the envelope glycoprotein sequences of HIV-1 and HIV-2 isolates showed a number of regions with varying degrees of conservation. However most of these were unconserved when comparison was also made to SIV sequences and only a single region of significant size was still conserved. This occurs in gp 120, immediately before the first hypervariable (V1) loop (9), and the conservation is shown in Table 2. The undecapeptide is absolutely conserved in all isolates of HIV and the changes in SIV are highly conservative, with only changes of T to S and V to I in most isolates (and L

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to I, T to N and L to Y in the, otherwise, highly divergent African mandrill isolate GB1).

The sequence of 11 amino acids appears to be unique to HIV and SIV, with no matches of more than 5 contiguous residues being found on searching the EMBO and SwissProt sequence data bases (or 6 residues, allowing gaps). The restricted sequence variation of this region of the otherwise highly variable gp 120 suggests that it might be essential for HIV viability and hence not be able to vary significantly and yet maintain infectious progeny virions.

Alignment of the sequences also showed a high degree of conservation of a second region, immediately after the second hypervariable (V2) loop (9) and disulphide-bridged to both cysteine residues in the first, uniquely conserved region (14), and the conservation is shown in Table 3. The conservation is greatest between the cysteine residues forming the disulphide bridges and each HIV Type is mutated only very conservatively, with the SIV isolates resembling one or other HIV Type. This further suggests that the antiparallel cross-linked peptides form a structure in gp 120 essential for viability of the virus.

Example 1

Based on this analysis, experiments were carried out using a synthetic peptide based on the highly conserved sequence of the invention.

All chemicals were of analytical grade and were used without further purification, unless otherwise mentioned.

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Peptide Synthesis

A peptide having the sequence KPCVKLTPLCVTLY (Seq. Id. No.4) was synthesised by Fmoc continuous flow solid phase synthesis using an automatic synthesiser (LKB Biolynx). Fmoc amino acid pentafluorophenyl esters were used as acylating species throughout, except for Thr where the ester of 2,3-dihydro-3-hydroxy-4-oxo-benzotriazine was preferred. Kieselguhr supported polydimethyl-acrylamide functionalised with the hydroxymethylphenoxy acetic acid linkage and norleucine as internal reference amino acid (18,19) was used as solid support.

The peptide was cleaved from the resin using trifluoroacetic acid/phenol/triethylsilane (23ml/1g/1ml for 500mg of peptide-resin assembly) which effected simultaneous cleavage of the tert-butyl based side chain protecting groups except for the acid stable AcM (acetamidomethyl) on the side chain of Cys. The resin was removed by filtration and washed with a little neat trifluoroacetic acid (TFA). The solvent was removed from the combined TFA filtrates by rotary evaporation under reduced pressure. The oily residue was dissolved in 0.1% aq. TFA (20ml) and extracted with diethyl ether (5 x 20ml) and the solvent removed from the combined aqueous phases by freeze drying to yield a white powder (20.9 umols, 76% yield).

The peptide was analysed by HPLC after synthesis and found to be at least 98% pure and was therefore used, without further purification, for coupling to KLH for immunisation of rabbits.

Antiserum production

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The peptide was coupled, in about 30-fold molar excess, to KLH, essentially by the protocol of Bassiri and Utiger (20) as modified by Kiberstis et al. (21). Since we were particularly concerned to present the peptide in a condition as near to that in the native protein as possible, the Cys residues were left as the S-acetamidomethyl derivative, which was chosen to approximate to one half of a disulphide bond, and the epsilon-amino groups of the Lys residues were reversibly protected during the coupling reaction, which would otherwise have converted them to hydroxyl groups, by modification with 2,3-dimethylmaleic anhydride (10-fold molar excess) at pH 9.0, prior to coupling, with subsequent unblocking at pH 6.5, 20°C, for 8h (22).

Three New Zealand white rabbits (known as Ping, Pong and Pang) (conventionally bred from Rosemead Rabbits Ltd., UK) were immunised with the equivalent of 0.6mg peptide in Freund's complete adjuvant, by multiple subcutaneous injections and subsequently given 2 boosts subcutaneously with the equivalent of 0.3mg peptide in incomplete Freund's adjuvant. Preimmune and post-immunisation blood samples were taken, together with the final immune blood, and sera prepared by allowing the samples to clot at room temperature.

Immune responses were monitored by ELISA against the peptide bound to wells of microtitre plates, which were subsequently blocked with bovine serum albumin. Horse radish peroxidase-conjugated goat anti-rabbit antibody (DAKO Ltd.) was used as the second antibody and 1,2-phenylenediamine, dihydrochloride (DAKO Ltd.) as the substrate.

ELISA of the rabbit preimmune sera and also sera after immunisation showed that all 3 rabbits developed a response to the peptide as an immunogen, when presented coupled to keyhole limpet hemocyanin (Table 4). The final bleeds give enhancements (greater than or equal to 4-fold compared to preimmune serum) at dilutions of 1:6400 or 1:3200, suggesting that the peptide was immunogenic when presented as the conjugate on KLH.

Immunofluorescence study of binding of antisera to HIV-infected cells

Binding of antibodies in the rabbit sera to native gp 120 was assayed using confocal immunofluorescent microscopy (23) with the Bio-Rad MRC 600 confocal microscope, with fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit IgG (Sigma Chemical Company), as the second antibody to show the rabbit antibody. Positive control rabbit antiserum against intact gp120 was a kind gift of Dr Rod Daniels of the National Institute for Medical Research, London. The test cells were the human T-lymphoblastoid cell line, C8166, which is non-productively infected with human T-cell lymphotropic virus Type-1 (24). These were grown in RPMI/10% fetal calf serum and infected with HIV-1 BRU (25), HIV-1 SF2 (26), or else uninfected as a control. Cells (10^3 in 15ul) were transferred to the wells of poly-L-lysine treated microscope slides and allowed to bind for 30 min at room temperature, before being fixed with 4% glutaraldehyde then treated with 0.1% Triton X100 to permeabilise the plasma membranes. Antisera at appropriate dilutions in PBS were added and allowed to bind for 60min at room temperature before the cells were washed. Fluorescein-labelled second antibody

was then added (at the dilution recommended by the supplier) and allowed to bind for 60min before washing in PBS. The slides were then examined in the confocal microscope, to detect antibody binding, and appropriate specimens photographed.

Binding of immune sera to cells expressing gp 120 of HIV-1_{IIIB} was determined, using both preimmune sera and also uninfected cells as controls. Typical fields for one rabbit (Pang) are shown in Figure 1. In the upper panel of this Figure, cells were either not expressing gp 120 (a,b and f) or expressing (all other photographs) and were treated with either preimmune serum (a and e) or immune serum (all other photographs). In the lower panel of this Figure, all cells were expressing gp 120 and were treated with immune serum from one rabbit (Pang) either without peptide (i and m) or after incubation with peptide A (j and n), or from another rabbit (Ping) again without peptide (k and o) or after incubation with peptide A (l and p).

Figure 1 shows major binding only with the immune sera and gp 120 expressing cells, while low binding is seen for serum onto non-expressing cells or for preimmune serum onto infected cells. The distribution of binding of immune serum to the gp 120 expressing cells is very similar to that seen for control serum, from a rabbit immunised with gp 120, binding to expressing, but not non-expressing, cells. Moreover, the binding of immune serum to infected cells is blocked by the peptide, indicating that it is to a specific site on the gp 120.

Binding of the immune sera to HIV-derived material in

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infected cells was determined, using both preimmune sera and also uninfected cells as controls. Typical fields for one rabbit (Ping), with HIV-BRU, are shown in Figure 2. In this Figure, cells were either uninfected (a,b,e and f) or infected with HIV-1 BRU (c and d) or SF2 (g and h), while the serum was either preimmune (a, c, e and g) or the immune (b, d, f and h). Bar represents 25 um.

Figure 2 shows major binding only with the immune serum and infected cells, while low binding is seen for either serum onto uninfected cells or for preimmune serum on infected cells. The distribution of the binding of immune serum to the infected cells is similar to that seen for control serum, from a rabbit immunised with gp 120, binding to infected cells, but not uninfected cells. Repetition of these experiments with cells infected with HIV-1 SF 2 showed very similar results (Figure 1 g and h), confirming that the reaction is independent of the HIV strain employed.

Results of similar tests with sera from the 2 further rabbits (Pong and Pang) and HIV-1 SF2 are shown in Figure 3. Figure 3a shows results using either preimmune serum (upper left) or immune serum (remaining pictures) from Pong with infected cells in each case, and Figure 3b shows results using immune serum from Pang, with either uninfected cells (upper left) or infected cells (remaining pictures). Bar represents 25 um.

Similar binding was seen with the sera from the rabbit Pong to cells infected with HIV-1 BRU or SF2 (Figure 3a). The third rabbit (Pang) again showed binding of its hyperimmune serum, but in this case the preimmune serum also bound to HIV-1 BRU-infected cells and so no

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conclusion can be drawn about the response. However, cells infected with HIV-1 SF 2 showed results very similar to those with the other rabbits, with both the preimmune and immune sera (Figure 3b) and, with this strain of virus, the preimmune serum did not bind and so binding could be shown to result from immunisation.

Figure 4 shows binding of sera from one rabbit (Pong) to cells infected with HIV-2. The cells were either uninfected (a and b) or infected with HIV-2_{CAM2}, and were treated with either preimmune (a and c) or immune serum (b and d). Again binding is seen only with the immune serum onto infected cells.

These results demonstrate that the highly conserved sequence of gp 120 in the form presented, i.e. coupled to keyhole limpet hemocyanin and with the cysteine residues in a configuration mimicking disulphide bridges and the epsilon-amino groups of the lysine residues maintained, gave a good immune response in the 3 rabbits tested. The resulting antisera bind to native gp 120 from a number of strains of HIV-1, and to HIV-2.

This extent of conservation, coupled with the surface accessibility of the epitope, suggests that this peptide may have some functional role in the native protein. One possible role could be in binding to a receptor additional to the well characterised CD4.

Example 2

Further experiments have been carried out using a peptide including the conserved sequence of the invention (A chain peptide) cross linked by disulphide bridges to a further

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peptide (B chain peptide) based on the cross linked sequences naturally occurring in gp 120.

The sequences of A chain peptide and B chain peptide (with cross-linking indicated) are as follows:

K P C V K L T P L C V T L Y	(A chain)
	(Seq.Id.No.4)
L I N C N R S A I K E S C P K V S F	(B chain)
	(Seq. Id.No.5)

Further experiments were carried out using a disulphide-bridged peptide based upon the sequences A and B of the invention.

Peptide Synthesis

Peptides having the sequences DQSLKPCVKLTPLCVTLY (A chain) (Seq.Id.No.6) and LINCNRSAIKESCPKVSF (B chain) (Seq.Id.No.5) were synthesised as described in Example 1, but with the cysteine residues protected by Acn on cysteine 7 and Trityl on cysteine 14 of the A chain, and by Trityl on cysteine 4 and Acn on cysteine 13 of the B chain. Trityl groups were removed from each peptide by acidolysis and the resulting cysteine in the A chain then reacted with 2-dipyridyl disulphide and gel filtered to remove excess reagent. The activated peptide was then reacted with 1.5-fold excess of B chain and the singly disulphide-bridged peptide purified on Whatman CM52 cation exchange resin.

The second disulphide-bridge was then formed specifically by reaction with 10 equivalents of I_2 in methanol. The

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peptide was finally purified by gel filtration.

Antiserum Production

The disulphide-bridged peptide was coupled to KLH exactly as described in Example 1, with the same protection for the lysine residues. Two rabbits (Tristan and König Marke) were immunised by subcutaneous injection of the KLH conjugate in Freund's complete adjuvant, followed by a single boost with the same conjugate in Freund's incomplete adjuvant. Subsequent boosts were with peptide alone in Freund's incomplete adjuvant.

Immune responses were analysed by ELISA against biotinylated peptide bound to streptavidin coated wells of microtitre plates. The development reagents were the same as those in Example 1.

ELISA of the rabbit preimmune sera and also sera after immunisation showed that both rabbits developed a response to the peptide as immunogen, when presented as described (Table 5). The final bleeds gave enhancements (greater than 10-fold compared to the preimmune sera) at dilutions of 1:16000.

Study of Virus Neutralisation by Antisera

Samples (50 tissue culture infective doses (TCID₅₀)) of HIV-1_{BRU} were incubated with 1:20 dilutions of either preimmune or immune sera from one rabbit (Tristan), or as controls with PBS or serum from a rabbit immunised with gp 120 from HIV-1_{BRU}, for 60 minutes at 37°C. The treated virus was then used to infect C8166 tissue culture cells and the course of infection followed by standard methods,

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measuring the virus present in the supernatant over the cells by the reverse transcriptase activity released by Triton X100, following the incorporation of ^3H -dTTP with poly-A as template and oligo-dT as primer.

Figure 5 shows that the virus in the supernatant rose about 1000 fold between days 7 and 12 after infection with the virus incubated with the control of PBS, while incubation of the virus with antiserum against gp 120 prevented any sign of infection. The preimmune serum did not affect the infection, but the immune serum was as effective in preventing infection as the anti-gp 120 control serum.

Immunisation with the disulphide-bridged peptide of the invention, by the method described above, therefore led to the presence of antibodies in the serum which would neutralise the virus infectivity. Moreover the sequence of the B chain in the immunogen is not identical to that of the equivalent peptide in HIV-1_{BRU} and yet neutralisation is found, suggesting that the overall peptide will lead to antibodies reacting with variants of HIV-1 or HIV-2, although better reaction with the specific HIV Types might be obtained with one peptide species for each as discussed above.

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Table 1

Abbreviations for amino acids

Amino acid	Three-letter abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Consensus 10 20
 DOSLKPCVKLTPLCVTLNC-C (Seq. Id. No. 7)

1 HIV-1: Z 6 -----T-
 2 HIV-1: HXB 2 -----S-K-T-
 3 HIV-1: ELI -----S-
 4 HIV-1: HAN -----K-T-
 5 HIV-1: CDC 45i -----T-
 6 HIV-1: BRVA -----H-
 7 HIV-1: JFL -----IN
 8 HIV-1: BRU -----S-K-T-
 9 HIV-1: JH 3 -----I-
 10 HIV-1: JR-CSF -----K-
 11 HIV-1: JR-FL -----K-
 12 HIV-1: ALA 1 -----H-I-
 13 HIV-1: JY 1 --N-----TN
 14 HIV-1: MAL -----TN
 15 HIV-1: MFA -----N-K-T-
 16 HIV-1: MN -----T-
 17 HIV-1: NDK -----T-
 18 HIV-1: NY 5 -----T-
 19 HIV-1: OYI -----D-T-
 20 HIV-1: RF -----T-
 21 HIV-1: SC -----TN
 22 HIV-1: SF 162 -----H-TN
 23 HIV-1: SF 2 -----T-
 24 HIV-1: SF 33 -----T-
 25 HIV-1: WMJ 2 -----I-
 26 HIV-1: Z2 Z6 -----I-
 27 HIV-1: Z 321 -----S-HN
 28 HIV-2: ST ET-I-----AMR-NS
 29 HIV-2: GH 1 ET-I-----AMS-NS
 30 HIV-2: ROD ET-I-----AMK-SS
 31 HIV-2: BEN ET-I-----AM--SR
 32 HIV-2: ISY ET-I-----M--NA
 33 HIV-2: NIH Z ET-I-----AM--TR
 34 HIV-2: D 194 ET-I-----AM--NI
 35 HIV-2: D 205 ET-I-----AM--SK

36 SIV: CPZ -----Q-SK
 37 SIV: MNE ET-I-----I-MK-NK
 38 SIV: SMM-PBJ ET-I-----I-MR-NK
 39 SIV: SMM-H4 ET-I-----IAMR-NK
 40 SIV: MM 239 ET-I-----S---I-MR-NK
 41 SIV: MM 251 ET-I-----S---I-MR-NK
 42 SIV: MM 142 ET-I-----S---I-MR-NK
 43 SIV: AGM 3 E-T-----S---IKMS-VE
 44 SIV: AGM 155 E-T-----S---IKM--VE
 45 SIV: TYO-1 E-TM-----S---IKM--VE
 46 SIV: MN-DGB1 -TI-----IN-Y--KMQ-QE

(IAMR is Seq. Id. No. 8)

(IKMS is Seq. Id. No. 9)

Table 3

Consensus			10	20	(Seq. Id. No. 10).
			LI-CNTSVITQACPKVSF		
1	HIV-1:	Z 6	--N----	A-----	
2	HIV-1:	HXB 2	-TS-----		
3	HIV-1:	ELI	--N----	A-----	
4	HIV-1:	HAN	--H--R-----		
5	HIV-1:	CDC 451	--N-----		
6	HIV-1:	BRVA	--S-----	T-	
7	HIV-1:	JFL	--N----	T-----	
8	HIV-1:	BRU	-TS-----		
9	HIV-1:	JH 3	--S-----	I--	
10	HIV-1:	JR-CSF	--S-----		
11	HIV-1:	JR-FL	--S-D-----	I--	
12	HIV-1:	ALA 1	--S-----		
13	HIV-1:	JY 1	--N----	A-----T-	
14	HIV-1:	MAL	--N-----	T-	
15	HIV-1:	MFA	--S-----		
16	HIV-1:	MN	--S-----	I--	
17	HIV-1:	NDK	--N-D--T-----	I--	
18	HIV-1:	NY 5	--N-D-----		
19	HIV-1:	OYI	--H----T-----	I--	
20	HIV-1:	RF	--H--S-----		
21	HIV-1:	SC	--N-----		
22	HIV-1:	SF 162	--N-----		
23	HIV-1:	SF 2	--H--R-----		
24	HIV-1:	SF 33	--H--S-----	T-----	
25	HIV-1:	WMJ 2	--N-----		
26	HIV-1:	Z2 Z6	--N----	A-----	
27	HIV-1:	Z 321	--N----	A-----	
28	HIV-2:	ST	MNH-----	ES-D-HYW	
29	HIV-2:	GH 1	MNH-----	ES-D-HYW	
30	HIV-2:	ROD	MNH-----	ES-D-HYW	
31	HIV-2:	BEN	MRH----	I-KES-D-HYW	
32	HIV-2:	ISY	MNH-----	ES-D-HYW	
33	HIV-2:	NIH Z	MNH-----	ES-D-HYW	
34	HIV-2:	D 194	MRH-----	KES-D-HYW	
35	SIV:	CPZ	I-N---TA-----	T--	
36	SIV:	MNE	MNH-----	QES-D-HYW	
37	SIV:	SMM-PBJ	MNH-----	QES-D-HYW	
38	SIV:	SMM-H4	MHH-----	QES-D-HYW	
39	SIV:	MM 239	MNH-----	QES-D-HYW	
40	SIV:	MM 251	MNH-----	QES-D-HYW	
41	SIV:	MM 142	MNH-----	QEC-D-DYW	
42	SIV:	AGM 3	M-H--D---KE--D-TYW		
43	SIV:	AGM 155	M-H--D---KE--D-TYW		
44	SIV:	TYO-1	M-H--D---KE--D-TYW		
45	SIV:	MN-DGB1	MNH--E--N-ED-Q-GLL		

Table 4. ELISA reactivities of sera from rabbits used for immunisation studies.

Serum dilution	No peptide added to well	Ping		Pang		Pong	
		Preimmune	Immune serum	Preimmune	Immune serum	Preimmune	Immune serum
1:3200	0.045	0.029	0.354	0.104	0.337	0.130	0.566
1:6400	0.051	0.034	0.183	0.052	0.210	0.089	0.363

Table 5. ELISA reactivities of sera from rabbits immunised with HB-AIDS2.

Serum dilution	No peptide added to well	Tristan		König Marke	
		Preimmune	Immune serum	Preimmune	Immune serum
1:2000	0.083	0.056	4.000	0.074	3.889
1:4000	0.076	0.059	4.000	0.067	2.556
1:8000	0.070	0.060	4.000	0.063	1.467
1:16000	0.083	0.081	2.817	0.077	0.880

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References

1. Ratner, L, Gallo, R C & Wong-Staal, F (1985) Nature (Lond.) 313, 636-637.
2. Clavel, F, Guyader, M, Guetard, D, Salle, M, Montagnier, L & Alizon, M (1986) Nature (Lond.) 324, 691-695.
3. Hahn, B H, Shaw, G M, Taylor, M E, Redfield, R R, Markham, P D, Salahuddin, S Z, Wong-Staal, F, Gallo, R C, Parks, E S & Parks, W P (1986) Science (Wash.) 232, 1548-1553.
4. Saag, M S, Hahn, B H, Gibbons, J, Li, Y, Parks, E S, Parks, W P & Shaw, G M (1988) Nature (Lond.) 334, 440-444.
5. Daniel, M D, Letvin, N L, King, N W, Kannagi, M, Sehgal, P K, Hunt, R D, Kanki, P J, Essex, M & Desrosiers, C (1985) Science (Wash.) 228, 1201-1204.
6. Franchini, G, Gurgo, C, Guo, H-G, Gallo, R C, Collati, E, Fagnoli, K A, Hall, L F, Wong-Staal, F & Reitz, M S J (1987) Nature (Lond.) 328, 539-543.
7. Chakrabarti, L, Guyader, M, Alizon, M, Daniel, M D, DeRosiers, R C, Tiollais, P & Sonigo, P (1987) Nature (Lond.) 328, 543-547.
8. Starcich, B R, Hahn, B H, Shaw, G M, McNeely, P D, Modrow, S, Wolf, H, Parks, E S, Parks, W P, Josephs, S F, Gallo, R C & Wong-Staal, F (1986) Cell 45, 637-648.

- 31 -

9. Modrow, S, Hahn, B H, Shaw, G M, Gallo, R C, Wong-Staal, F & Wolf, H (1987) J Virol. 61, 570-578.
10. Rusche, J R, Javaherian, K, McDanal, C, Petro, J, Lynn, D L, Grimaila, R, Langlois, A, Gallo, R C, Arthur, L O, Fischinger, P J, Bolognesi, D P, Putney, S D & Matthews, T J (1988) Proc. Nat. Acad. Sci. USA 85, 3198-3202.
11. Kuhnel, H, von Briesen, H, Dietrich, U, Adamski, M, Mix, D, Biesert, L, Kreutz, R, Immelmann, A, Henco, K, Meichsner, C, Andreesen, R, Gelderblom, H & Rubsamen-Waigmann, H (1989) Proc. Natl. Acad. Sci. USA 86, 2383-2387.
12. Tsujimoto, H, Hasegawa, A, Maki, N, Fukasawa, M, Miura, T, Speidel, S, Cooper, R W, Moriyama, E N, Gojobori, T & Hayami, M (1989) Nature (Lond.) 341, 539-541.
13. Davis, D, Stephens, D M, Willers, C - Lachmann, P J (1990) J Gen. Virol. 71, 2889-2898.
14. Leonard, C K, Spellman, M W, Riddle, L, Harris, R J, Thomas, J N and Gregory, T J (1990). J. Biol. Chem., 265, 10373-10382.
15. Davies, J S (1984) in Spec. Periodical Reps., Amino-Acids Peptides and Proteins, Roy. Chem. Soc. London.
16. Blundell, T, Sibanda, B L and Pearl, L (1983) Nature (Lond.), 304, 273-275.
17. Sibanda, B L, Hemmings, A M and Blundell, T L (1985)

- 32 -

in "Aspartic Proteinases Their Inhib.", Proc FEBS Adv. Course 1984, pp 339-349. Ed. Kostka, V Publ. by de Gruyter, Berlin.

18. Atherton, E & Sheppard, R C (1989) "Solid Phase Peptide Synthesis: A practical approach." (Oxford University Press, Oxford).

19. Dryland, A & Sheppard, R C (1988) Tetrahedron 44, 859.

20. Bassiri, R M & Utiger, R D (1972) Endocrinology 90, 722-727.

21. Kiberstis, P A, Pessi, A, Atherton, E, Jackson, R, Hunter, T & Zimmern, D (1983) FEBS Lett. 164, 355-360

22. Butler, P J G & Hartley, B S (1972) in "Methods in Ezymology" (ed. C W Hirs and S N Timasheff), Vol. 25, pp 191-199 (Academic Press, New York and London).

23. White, J G, Amos, W B & Fordham, M (1987) J. Cell Biol. 105, 41-48.

24. Salahuddin, S Z, Markham, PD, Wong-Staal, F, Franchini, G, Kalyanaraman, V S & Gallo, R C (1983) Virology 129, 51-64.

25. Wain-Hobson, S, Sonigo, P, Danos, O, Cole, S & Alizon, M (1985) Cell 40, 9-17.

26. Sanchez-Pescadoe, R, Power, M D, Barr, P J, Steimer, K S, Stempien, M M, Brown-Shimer, S L, Gee, W W, Renard,

- 33 -

A, Randolph, A, Levy, J A, Dina, D & Luciw, P A (1985)
Science (Wash.) 227, 484-492.

SEQUENCE LISTING

1. GENERAL INFORMATION:

1. APPLICANT:

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(E) COUNTRY: United Kingdom
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(ii) TITLE OF INVENTION: Developments relating to human immunodeficiency viruses

(iii) NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
(EPO)

2. INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

WO 94/02614

PCT/GB93/01503

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val
1 5 10

(1) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Asn Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asp Ser Thr Ala
1

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(ii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Lys	Pro	Cys	Val	Lys	Leu	Thr	Pro	Leu	Cys	Val	Thr	Leu	Tyr
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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Leu	Ile	Asn	Cys	Asn	Arg	Ser	Ala	Ile	Lys	Glu	Ser	Cys	Pro
1				5					10				

Lys	Val	Ser	Phe
15			

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys
1 5 10

Val Thr Leu Tyr
15

(2) INFORMATION FOR SEQ ID NO: 7:

- (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asp Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys
1 5 10

Val Thr Leu Asn Cys
15

(2) INFORMATION FOR SEQ ID NO: 8:

- (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Ala Met Arg
1

(2) INFORMATION FOR SEQ ID NO: 9:

- (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ile Lys Met Ser
1

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro Lys Val Ser
1 5 10

Phe
15

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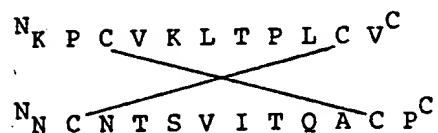
CLAIMS

1. A molecule comprising a peptide having the amino acid sequence lysine-proline-cysteine-valine-lysine-leucine-threonine-proline-leucine-cysteine-valine (Seq.Id.No.1), wherein each cysteine residue is disulphide bridged to a further cysteine residue or is derivatised to simulate part of a disulphide bond, or functionally equivalent variants of such a peptide which mimic the immunogenic behaviour of an epitope of gp120 of HIV env.

2. A molecule according to claim 1, wherein each cysteine residue is derivatised as a S-acetamidomethyl derivative.

3. A molecule according to claim 1, cross-linked to a further peptide sequence.

4. A molecule according to claim 3, cross-linked as follows:



5. A molecule according to any one of claims 1 to 4, wherein the or each peptide sequence is modified by one or more individual amino acids having been substituted by amino acids having comparable properties.

6. A molecule according to any one of claims 1 to 5, wherein the or each peptide sequence is modified by one or more of the normal peptide linkages having been substituted by isosteric replacements.

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7. A molecule according to any one of claims 1 to 6, wherein the or each peptide sequence is modified by one or both pairs of disulphide-bridged cysteine residues having been substituted by a cystine analogue.

8. A molecule according to any one of claims 1 to 7, wherein the or each peptide sequence is modified by one or more of the amino acids having been replaced by a "retro-inverso" amino acid.

9. A molecule according to any one of claims 1 to 8, wherein the or each peptide sequence is modified by one or more non-essential amino acids having been deleted.

10. A molecule according to any one of claims 1 to 9, wherein the or each peptide sequence is modified by inclusion of one or more additional amino acids not significantly affecting function.

11. A DNA molecule coding for a peptide molecule in accordance with any one of the preceding claims.

12. A molecule according to claim 11, incorporated into a suitable expression vector.

13. A vaccine against AIDS and AIDS related conditions, comprising a molecule in accordance with any one of claims 1 to 10.

14. A vaccine according to claim 13, wherein the molecule of any one of claims 1 to 10 is linked to a carrier molecule.

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15. A vaccine according to claim 13, wherein the molecule of any one of claims 1 to 10 is presented as a live vaccine.

16. A method for the prophylaxis or treatment of AIDS or related conditions, comprising administering an effective amount of a molecule in accordance with any one of claims 1 to 10.

17. A pharmaceutical composition containing, as an active ingredient, a molecule in accordance with any one of claims 1 to 10, possibly in association with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.

18. Use of a molecule in accordance with any one of claims 1 to 10, for the preparation of a medicament for the therapy or prophylactic treatment of AIDS or related conditions.

19. A molecule that binds to the molecule of any one of claims 1 to 10, particularly an antibody, an antigen binding site of an antibody or a structural analog thereof.

20. Use of a molecule which binds to the molecule of any one of claims 1 to 10 for therapeutic or diagnostic purposes.

AMENDED CLAIMS

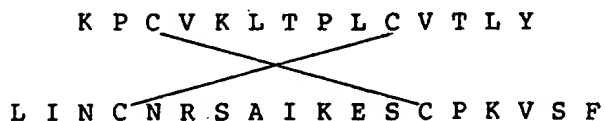
[received by the International Bureau on 20 December 1993 (20.12.93) ;
original claims 1-20 replaced by amended claims 1-21 (4 pages)]

1. A molecule comprising a peptide having the amino acid sequence lysine-proline-cysteine-valine-lysine-leucine-threonine-proline-leucine-cysteine-valine (Seq.Id.No.1), wherein each cysteine residue is disulphide bridged to a further cysteine residue or is derivatised to simulate part of a disulphide bond, or functionally equivalent variants of such a peptide which mimic the immunogenic behaviour of an epitope of gp120 of HIV env.
2. A molecule according to claim 1, wherein each cysteine residue is derivatised as a S-acetamidomethyl derivative.
3. A molecule according to claim 1, cross-linked to a further peptide sequence.
4. A molecule according to claim 3, cross-linked as follows:

$$\begin{array}{ccccccccccc}
 \text{N} & & & & & & & & & & \\
 \text{K} & \text{P} & \text{C} & \text{V} & \text{K} & \text{L} & \text{T} & \text{P} & \text{L} & \text{C} & \text{V}^{\text{C}} \\
 & & & & & & & & & & \\
 \text{N} & & & & & & & & & & \\
 \text{N} & \text{C} & \text{N} & \text{T} & \text{S} & \text{V} & \text{I} & \text{T} & \text{Q} & \text{A} & \text{C} & \text{P}^{\text{C}}
 \end{array}$$
5. A molecule according to any one of claims 1 to 4, wherein the or each peptide sequence is modified by one or more individual amino acids having been substituted by amino acids having comparable properties.
6. A molecule according to claim 5, comprising the following sequences, cross-linked as indicated:

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K P C V K L T P L C V T L Y
L I N C N R S A I K E S C P K V S F



7. A molecule according to any one of claims 1 to 6, wherein the or each peptide sequence is modified by one or more of the normal peptide linkages having been substituted by isosteric replacements.

8. A molecule according to any one of claims 1 to 7 wherein the or each peptide sequence is modified by one or both pairs of disulphide-bridged cysteine residues having been substituted by a cystine analogue.

9. A molecule according to any one of claims 1 to 8, wherein the or each peptide sequence is modified by one or more of the amino acids having been replaced by a "retro-inverso" amino acid.

10. A molecule according to any one of claims 1 to 9, wherein the or each peptide sequence is modified by one or more non-essential amino acids having been deleted.

11. A molecule according to any one of claims 1 to 10, wherein the or each peptide sequence is modified by inclusion of one or more additional amino acids not significantly affecting function.

12. A DNA molecule coding for a peptide molecule in accordance with any one of the preceding claims.

13. A molecule according to claim 12, incorporated into a suitable expression vector.

14. A vaccine against AIDS and AIDS related conditions, comprising a molecule in accordance with any one of claims 1 to 11.

15. A vaccine according to claim 14, wherein the molecule of any one of claims 1 to 11 is linked to a carrier molecule.

16. A vaccine according to claim 14, wherein the molecule of any one of claims 1 to 11 is presented as a live vaccine.

17. A method for the prophylaxis or treatment of AIDS or related conditions, comprising administering an effective amount of a molecule in accordance with any one of claims 1 to 11.

18. A pharmaceutical composition containing, as an active ingredient, a molecule in accordance with any one of claims 1 to 11, possibly in association with one or more pharmaceutically acceptable adjuvants, carriers and/or excipients.

19. Use of a molecule in accordance with any one of claims 1 to 11, for the preparation of a medicament for the therapy or prophylactic treatment of AIDS or related conditions.

20. A molecule that binds to the molecule of any one of claims 1 to 11, particularly an antibody, an antigen binding site of an antibody or a structural analog thereof.

21. Use of a molecule which binds to the molecule of any one of claims 1 to 11 for therapeutic or diagnostic purposes.

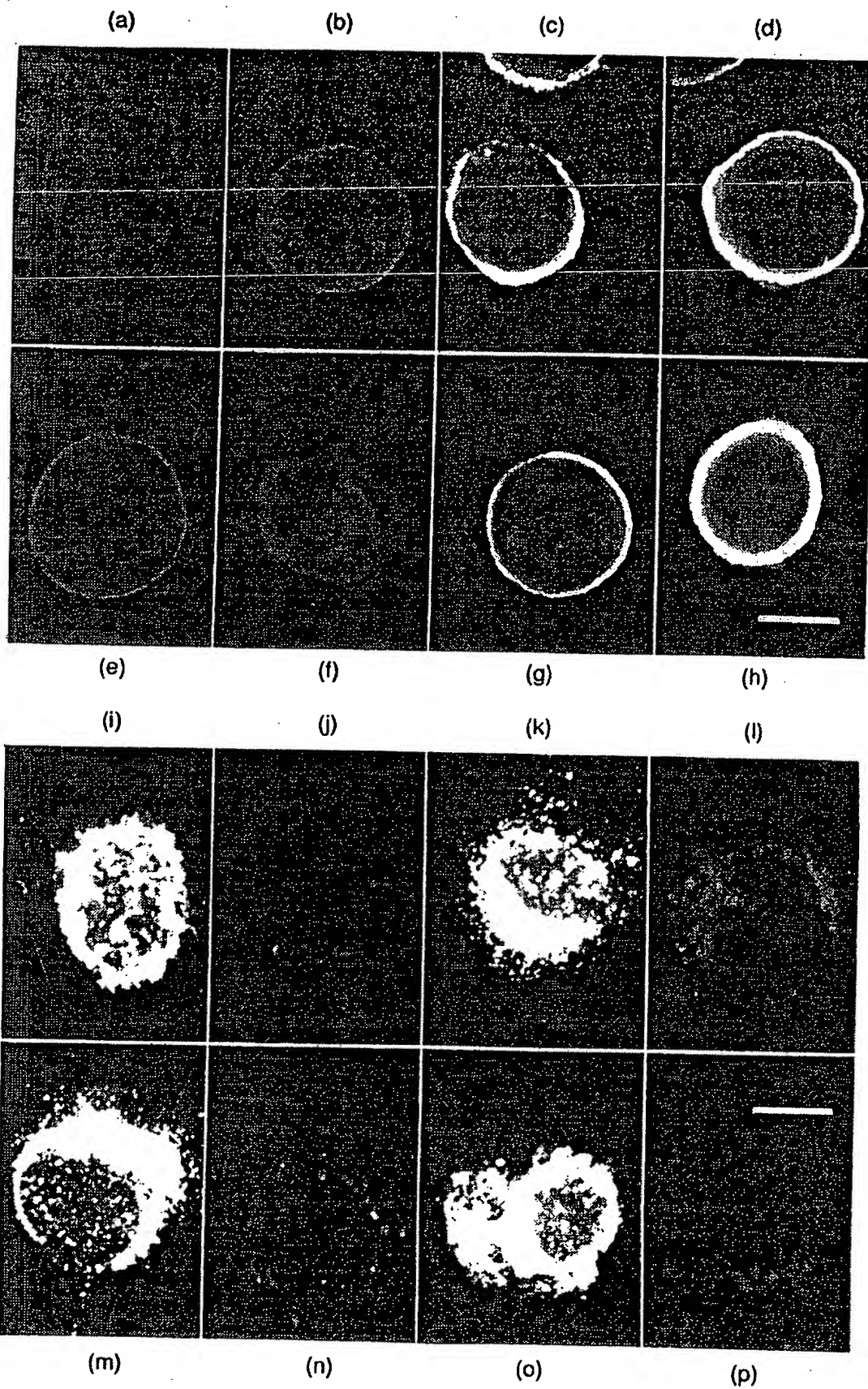


FIG 2

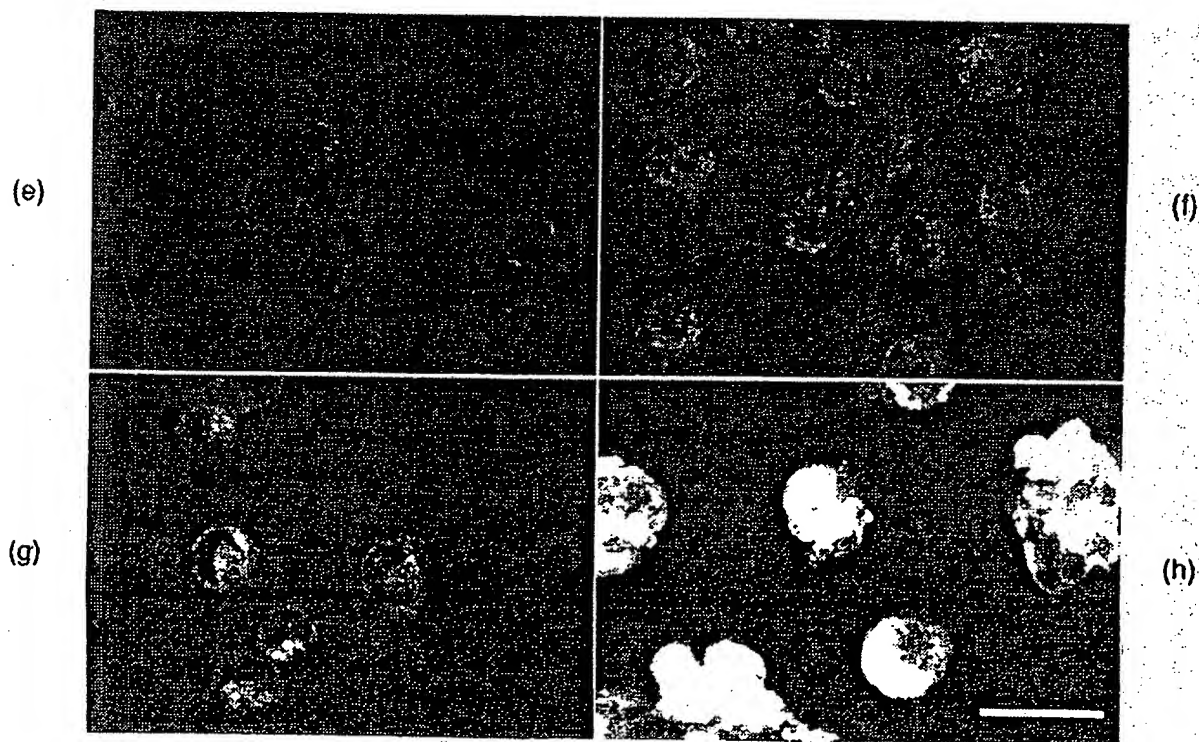
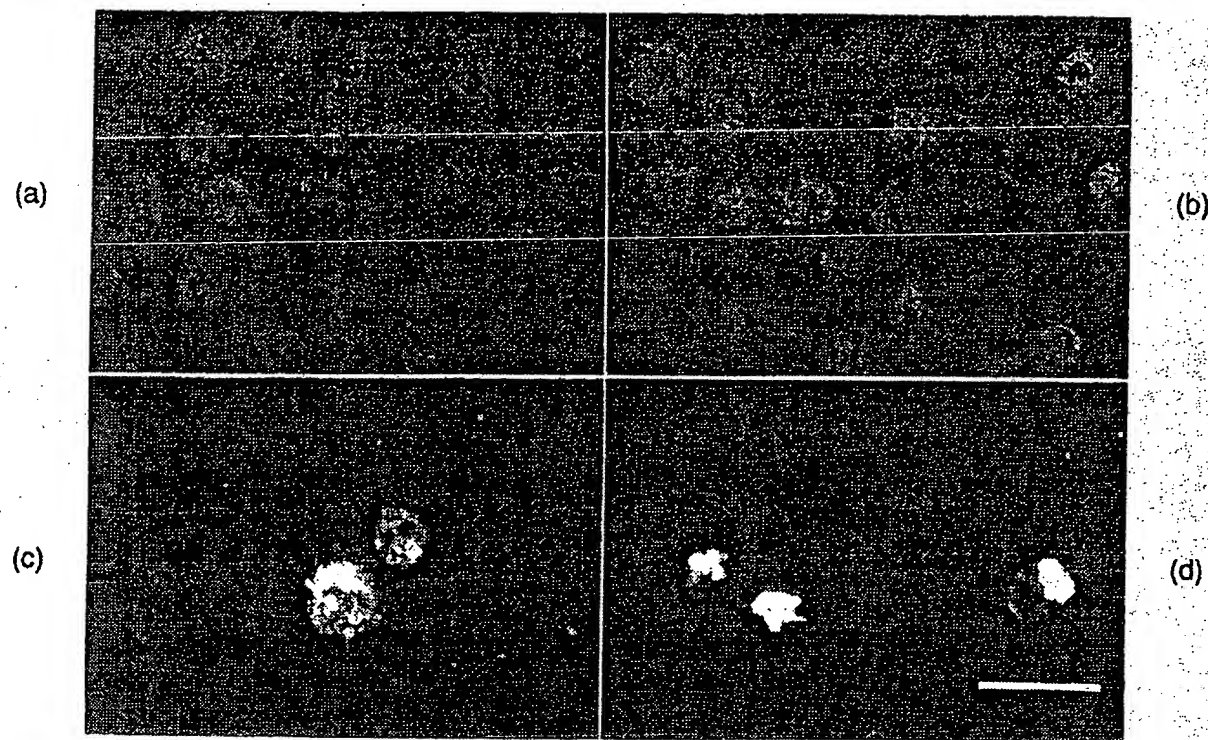


FIG 3

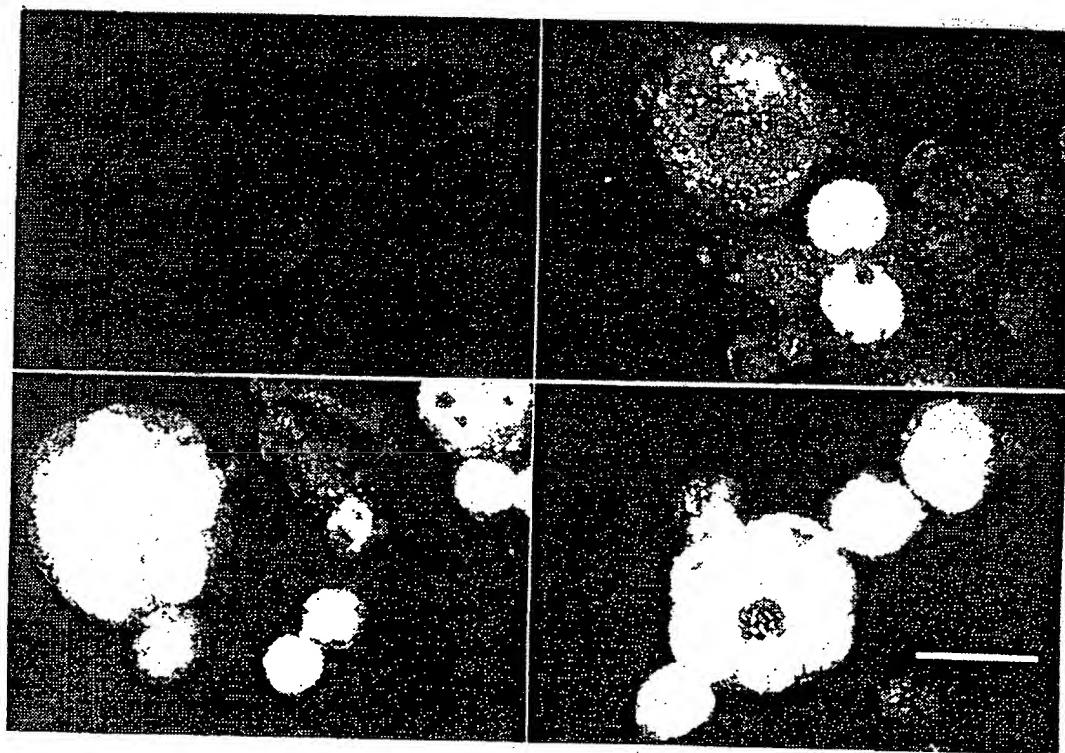
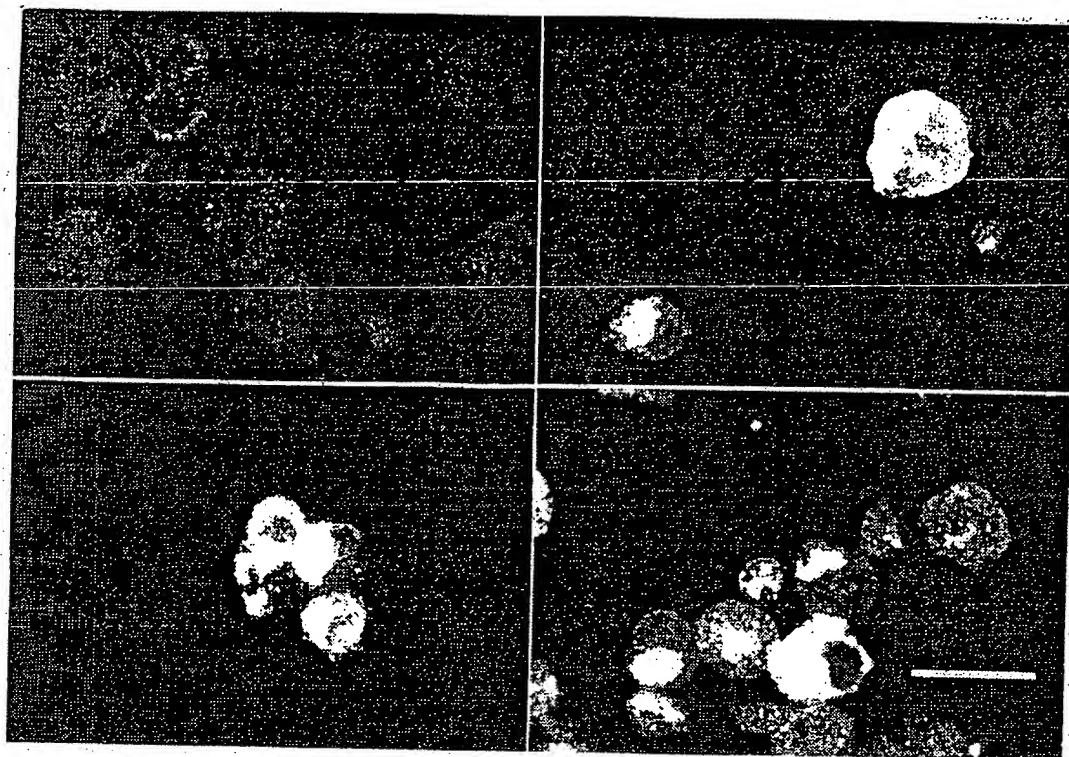


FIG 4

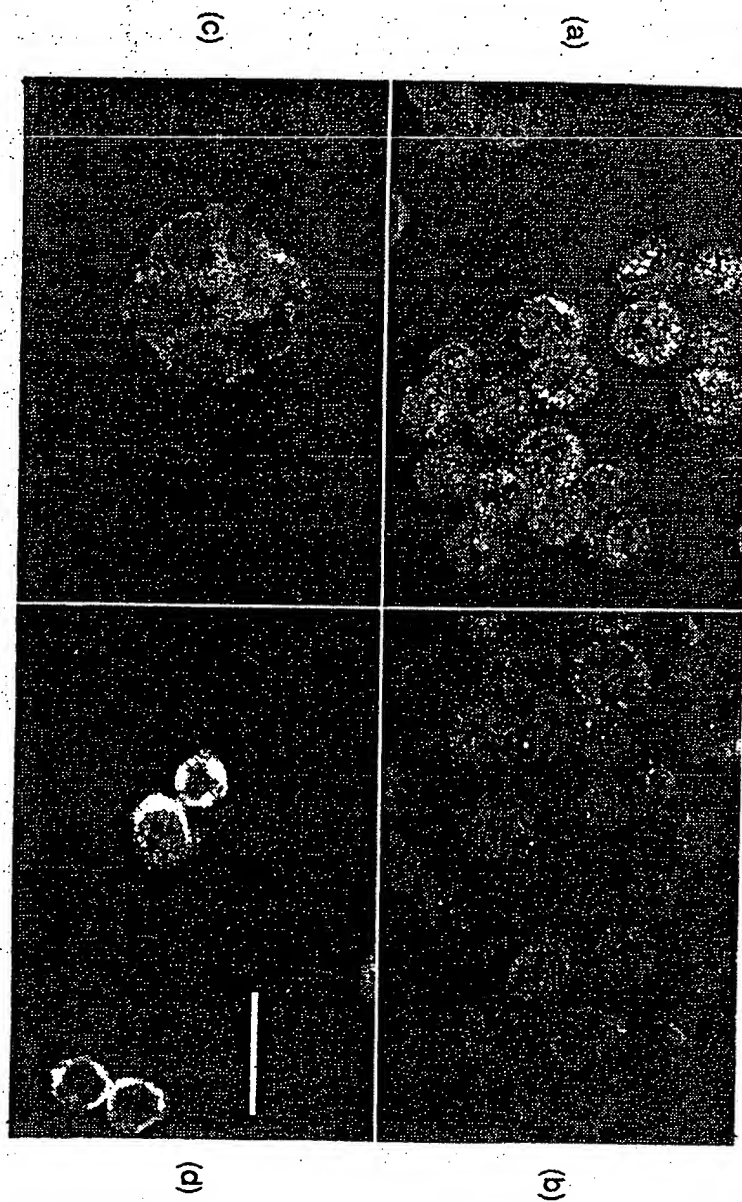
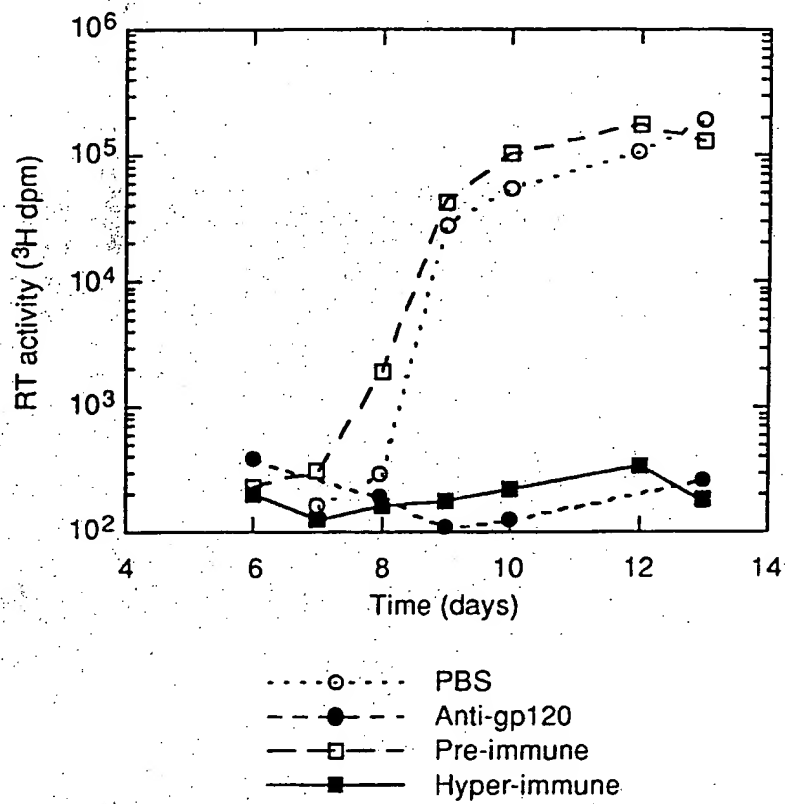


FIG 5



INTERNATIONAL SEARCH REPORT

Int. application No.

PCT/GB93/01503

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 16 and 20 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/GB 93/01503

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0298633	11-01-89	AU-B- 611457	13-06-91
		AU-A- 1957288	19-01-89
		EP-A- 0371046	06-06-90
		WO-A- 8810267	29-12-88
		JP-T- 2503916	15-11-90
		OA-A- 9152	31-10-91

EP-A-0498905	19-08-92	NONE	

EP-A-0371817	06-06-90	AU-B- 623949	28-05-92
		AU-A- 4576789	07-06-90
		JP-A- 2209891	21-08-90
